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MARKER FOR PREDICTING GRAFT-VERSUS-HOST DISEASE AND UTILIZATION OF THE SAME
MARKER ZUR VORHERSAGE EINER GRAFT-VERSUS-HOST-ERKRANKUNG UND DESSEN NUTZUNG
MARQUEUR DE PRÉVISION DE LA MALADIE DU GREFFON CONTRE L’HÔTE ET UTILISATION

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to a method of assaying a DNAM-1 level associated with the onset of acute graft-versus-host disease as a graft-versus-host disease predicting marker. Described is also a kit therefor and a molecular targeted therapy using the method. Therefore a test method is described using a graft-versus-host disease predicting marker for providing data helpful in predicting or estimating the risk of acute graft-versus-host disease by determining the DNAM-1 level in a blood specimen. Further, a kit therefor and a molecular targeted therapy using an anti-DNAM-1 antibody are described.

BACKGROUND ART

[0002] Blood plays a very important role in life-supporting by delivering oxygen to all parts of the body, or by attacking or protecting against nonself that has invaded the body. Blood contains three types of blood components: red blood cells, white blood cells and platelets. The white blood cells have a major and important function to protect a living body against nonself that has invaded the body, and they are classified into neutrophils, eosinophils, basophils, monocytes and lymphocytes. Accordingly, reduction in number of white blood cells disenables a living body to attack or eliminate pathogens that have entered the body, resulting in easy infection.

[0003] Leukemia is a disease in which white blood cells are malignantly transformed and proliferate mainly in bone marrow. Leukemia is subdivided into chronic leukemia and acute leukemia depending on the length of time in which it progresses when untreated, and each form is further divided into myeloid leukemia and lymphoblastic leukemia. Advanced leukemia destroys hematopoietic system and causes abnormalities such as reduction of normal white blood cells or platelets and anemia. And symptoms such as easy infection and bleeding tendency become clinically evident. When such hematopoietic dysfunction becomes worse, maintaining life will be threatened. Thus, leukemia is a fatal disease if untreated. Malignant lymphoma is a disease in which neoplastic lymphocytes proliferate mainly in lymph nodes, and is divided into Hodgkin lymphoma and non-Hodgkin lymphoma. Prognoses are varied depending on the lymphoma form, and a non-negligible number of cases result in death.

[0004] Hematopoietic tumors such as leukemia and malignant lymphoma have been addressed by killing tumor cells or normalizing the bone marrow function. Hematopoietic stem cell transplantation is one of such treatments. Each hematopoietic stem cell possesses capability to produce mature blood cells such as red blood cells and white blood cells, in other words, hematopoietic stem cells are multilineage potential, and they also have the potential to self-renew. Thus, they are mother cells for all the hematopoietic cells. Normal hematopoietic function may be recovered by eradicating tumor cells such as leukemia cells and malignant lymphoma cells by intense regimen of chemotherapy and radiation therapy and thereafter by transplanting hematopoietic stem cells.

[0005] Graft-versus-host disease that occurs after blood transfusion or stem cell transplantation is a serious side effect that should be addressed. Of the hematopoietic stem cell transplantations, allogeneic hematopoietic stem cell transplantation is often complicated with acute graft-versus-host disease (referred to as GVHD herein-after) as an early complication. Acute GVHD has a mechanism wherein activated T cells present in a graft from a donor recognize human leukocyte antigens (HLA) that express on the surface of white blood cells of a recipient (patient) as foreign and thereby the cytotoxic donor T cells attack cells of the recipient as foreign (Non-Patent Document 1). Patients having acute GVHD develop fever, skin rashes on limbs, face and trunk, liver damages with increased bilirubin, and digestive symptoms such as stomachache and diarrhea. GVHD is generally diagnosed by pathological examination on biopsy specimens of skin, liver and large intestine, but pathological findings are frequently not typical. Further, obtaining pathological materials is often difficult. Thus, there are many cases where this disease is diagnosed by clinical manifestations (Non-Patent Document 2). Okoshi et al (Cong. Jap. Soc. Clin. Hematology, PS-3-74 (2005)) reported that the number of NKT cells is reduced in patients with acute and chronic GVHD and investigate CD226 expression on NKT cells in patients after allogeneic hematopoietic stem cell transplantation. Further, in WO 00/69897, it is disclosed that inhibiting PDZ association with DNAM-1 will inhibit Fyn association with DNAM-1.

[0006] A general choice for early treatment of acute GVHD is administration of steroids. Steroids are effective for acute GVHD but may cause serious side effects such as infection with bacteria and viruses, and therefore use thereof must be considered carefully. On the other hand, delay in starting the treatment of acute GVHD can invite a life-threatening result. Due to this dilemma, how to diagnose acute GVHD in clinical cases is a critical problem that influences the success of transplantation. At present, there are no methods capable of predicting the onset of graft-versus-host disease after bone marrow transplantation.

[0007] It is demanded that data should be provided which are useful as direct evidence in the diagnosis of acute GVHD. Clinical experts expect that an index is represented to allow for primary linking of a target identified acute GVHD with physiological and biochemical changes associated with immune responses, based on statistical significance. Further, there is a demand for data contributing to the prediction of probability of disease development and the estimation of risk of the development. However, no test methods for providing such data have been established for this disease. Thus, development of such
It has been found that DNAM-1 is involved in the present invention and acute GVHD in clinical specimens. Determination exceeding this range is evaluated to be abnormally high from control values of healthy individuals, and a concentration exceeding this range is evaluated to be abnormally high.

DISCLOSURE OF THE INVENTION

As described above, the diagnosis standards of acute GVHD are frequently dependent on surface symptoms such as fever and skin rashes on patients and have less objectivity. Further, current diagnosis methods do not have specificity for acute GVHD, and identifying the disease from other disorders is difficult. In view of the above circumstances, the present inventors studied the correlation between DNAM-1 found by an inventor of the present invention and acute GVHD in clinical specimens. It has been then found that DNAM-1 is involved in the mechanism of acute GVHD development. This finding has established the significance of DNAM-1 in blood as a predicting marker that is specific for acute GVHD. The present inventors have thus developed acute GVHD-specific test methods capable of providing data useful in predicting the development of the disease, and kits for the methods, and GVHD molecular targeted therapy and pharmaceutical preparations for treating or preventing GVHD are also described herein.

It is an object of the present invention to provide a test method and a kit thereof selectively used in the prediction of acute GVHD development, which method and kit give data for predicting the probability of acute GVHD development or estimating the risk of the development.

It is another object of the invention to provide a pharmaceutical composition (preparation) for use in molecular targeted therapy wherein DNAM-1 in a GVHD patient or a recipient that is a possible patient is used as a target molecule and GVHD is treated or prevented by administering an anti-DNAM-1 neutralizing antibody.

The present invention therefore provides a test method for predicting acute GVHD development comprising determining the soluble DNAM-1 concentration in blood collected from a transplant patient and examining temporal changes of said concentration wherein the soluble DNAM-1 concentrations in blood are classified according to a normal range that is within Mean + 2SD from control values of healthy individuals, and a concentration exceeding this range is evaluated to be abnormally high. A test method for dealing with probability of GVHD development is described which comprises determining the DNAM-1 concentration in blood collected from a transplant patient and examining temporal changes of the concentration to provide data for predicting the probability of development of acute graft-versus-host disease (GVHD) or for estimating the risk of the development.

The data may include classified blood DNAM-1 concentrations on the basis of a normal range that is within Mean + 2SD calculated from control values of healthy individuals, and a concentration exceeding this range is evaluated to be abnormally high.

The data may be preferably shown as a time-series profile of the blood DNAM-1 concentrations.

The time-series profile may be shown in sections and the sections contain time points at least two of which have a higher DNAM-1 concentration than that of an immediately previous time point and which do not show a decreasing change.

The transplant patient may be a bone marrow or peripheral, hematopoietic stem cell transplantation patient.

It is preferable that the data are mainly collected from a patient who has had allogeneic hematopoietic stem cell transplantation and are used to help prognosis management.

A test method of the invention provides a time-series profile showing soluble blood DNAM-1 concentrations that is used for prognosis management for a patient who is under treatment of acute GVHD or that is used for determination of therapeutic effects for GVHD.

The blood DNAM-1 concentration may be determined by ELISA method using a Tx25 anti-DNAM1 monoclonal antibody.

The present invention also relates to the use of a hit which comprises at least a Tx25 anti-DNAM1 monoclonal antibody, a human DNAM-1/Fc chimeric protein and an ELISA tool and which is used to determine the blood DNAM-1 concentrations by performing an ELISA method using the Tx25 anti-DNAM1 monoclonal antibody to create the time-series profile.

A pharmaceutical preparation for use in treating or preventing GVHD according to the present invention comprises an anti-DNAM-1 neutralizing antibody. The anti-DNAM-1 antibody is preferably a monoclonal antibody.

Preferably, the anti-DNAM-1 antibody is a humanized DAM-1 antibody that is expressed by a human DNAM-1 gene introduced in a DNAM-1 knockout mouse.

The pharmaceutical preparation for use in treating or preventing GVHD may comprise at least a pharmaceutically acceptable excipient, carrier or stabilizing agent, and may further comprise a medicinal substance as an effective component for GVHD treatment.

The present invention also encompasses a use of a neutralising anti-DNAM-1 antibody in production of a pharmaceutical preparation for use in treating or preventing GVHD by administering the anti-DNAM-1 antibody to blood DNAM-1 of a GVHD patient or a recipient.
that is a possible patient, as a target molecule.

[0025] Molecular targeted therapy is also described herein where blood DNAM-1 of a GVHD patient or a recipient that is a possible patient is used as a target molecule and GVHD is treated or prevented by administering an anti-DNAM-1 antibody or an analogue thereof that is a neutralizing antibody.

ADVANTAGES OF THE INVENTION

[0026] The test methods of the invention provide the most reliable data for predicting the probability of development of acute graft-versus-host disease (GVHD) or for estimating the risk of the development.

[0027] The data provided by the test methods of the invention are based on findings from immunopathology dealing with the probability of GVHD development, and are presented in an appropriate and convenient format.

[0028] With the data provided by the test methods of the invention, the probability of GVHD onset is predicted and early treatment of GVHD is possible. Therefore, a decrease in mortality from acute GVHD and improved accomplishments of bone marrow transplantation are expected.

[0029] The test methods of the invention are very useful in obtaining data for use in preliminary estimation of the risk of GVHD development, its early diagnosis, and prognosis management after hematopoietic stem cell transplantation from bone marrow or the like.

[0030] The pharmaceutical preparations for use in treating or preventing GVHD according to the invention comprise a neutralizing antibody specific for DNAM-1 involved in GVHD, and can effectively prevent or treat GVHD complication by being administered to hematopoietic stem cell transplantation patients.

Detailed description of the invention

[0031] In the specification, GVHD is defined as a disease in which lymphocytes and the like in a graft transplanted into a host of different histocompatibility antigen type, engraft in the host, and recognize host tissues as foreign and attack the tissues. In this case, the "host" is a recipient (transplant patient) who has received blood infusion or transplantation. The present invention is mainly targeted at a human and is suitably used for human patients. However, the invention may be used for non-human animals in which at least antibody formation by immune reactions is observed. The term "acute" is used herein as an antonym for "chronic".

[0032] Described below are test methods, kits for performing the methods, and DNAM-1 molecular targeted therapy for GVHD.

Test methods

[0033] The test methods of the invention deal with a probability of development of acute graft-versus-host disease (GVHD). In the methods, data for predicting the probability of development of acute graft-versus-host disease (GVHD) or for estimating the risk of the development are provided by determining the DNAM-1 concentration in blood and examining temporal changes of the concentration.

[0034] The test method includes measuring the blood DNAM-1 concentration in a specimen and statistically analyzing the data obtained. In the method, the specimen is serum prepared from blood. The blood is a blood specimen collected from a transplant patient who has received blood infusion or stem cell transplantation likely complicated with acute GVHD. In particular, the blood specimen is collected from a leukemia patient who has received bone marrow or peripheral, hematopoietic stem cell transplantation. Such patients are likely to develop acute GVHD after transplantation, and the risk of the development can be predicted. The blood may be collected from any parts of the body without limitation. The blood may be collected in an amount enough to ensure a required amount of serum for performing the ELISA method as described later, and the blood amount may be preferably 1 to 2 ml. The timing and number of blood collection may be referred to doctors for decision, but the blood may be preferably collected on a plurality of days in view of the purpose of the test. Correlation between DNAM-1 molecule and acute GVHD DNAM-1 is a membrane protein discovered by Shibuya who is an inventor of the present invention (Shibuya A, et al., Immunity, 1996). DNAM-1 (CD226) is strongly expressed on activated T cells and NK cells having a cytotoxicity; they recognize ligands expressed on human tumor cells or the like and adhere thereto, playing an important role in inducing cytotoxicity against target tumor cells (Non-Patent Document 2). Part of the DNAM-1 molecules that have become more strongly expressed on the activated T cells are liberated from cell membranes and are released as soluble DNAM-1 in the blood stream. Therefore, the soluble DNAM-1 molecules that increase in blood of a recipient originate from white blood cells of a donor.

[0035] When T cells attack foreign cells or tumor cells, they adhere to the target cells and are activated, which initiates acute GVHD. In this process, cell adhesion molecules become involved as essential signal transducing molecules. Known adhesion molecules include cadherin family members bonding identical cells, integrin family members mainly expressed on white blood cells and involved in the bonding between cells or between a cell and an extracellular matrix, immunoglobulin family members playing roles in the bonding of cells, selectin family members and sialomucin family members involved in cell migration, and link family members involved in the bonding with an extracellular matrix. DNAM-1 is an adhesion molecule belonging to the immunoglobulin family. The present inventors advocate that this adhesion molecule when strongly expressed on activated T cells is involved in some way in the mechanism of acute GVHD development. In detail, DNAM-1 is involved in the bonding where-
in donor T cells in a graft perceive recipient’s white blood cells as nonself and are bonded thereto, with DNAM-1 recognizing a ligand expressed on the target cells and adhering thereto. In this process, DNAM-1 transduces an activation signal and induces cytotoxicity of the T cells.

Researches by the present inventors have demonstrated that soluble DNAM-1 in blood contributes to objective and specific diagnosis of acute GVHD. In detail, as will be demonstrated in Examples later, an increase of the soluble DNAM-1 level in blood has been shown to be closely associated with the onset of acute GVHD. Acute GVHD patients had higher blood DRAM-1 concentrations than before the onset of the disease. The inventors also confirmed that the blood DNAM-1 concentration did not increase in other blood diseases except acute GVHD. This indicates that the soluble DNAM-1 level in blood specifically changes with developing acute GVHD, but the level stays within the normal range with other blood diseases.

Accordingly, DNAM-1 molecules in blood are to be a specific predicting marker for acute GVHD onset, and detecting an increasing tendency of the level or a sign thereof enables prediction of the disease development. Thus, following temporal changes of the soluble DNAM-1 level in blood is an advantageous prognosis management after hematopoietic stem cell transplantation from bone marrow or the like. Accordingly, it is preferable that the data provided by the test methods of the invention are mainly collected from a patient who has had allogeneic hematopoietic stem cell transplantation and are used to help prognosis management. This is because allogeneic hematopoietic stem cell transplantation in which stem cells are offered by a related donor (a parent or brother) has much higher probability of acute GVHD development than autologous hematopoietic stem cell transplantation, as will be described in Example 1 later. This fact is based on inherent characteristics of immune system that strictly identifies self from nonself by HLA types.

In the test methods of the invention, the blood DNAM-1 concentration may be determined by any methods without limitation. A method capable of high precision measurement even with a trace amount of a specimen is preferable. In general, an analytical method using an antibody, preferably a monoclonal antibody, specific for DNAM-1 molecules is desirable because of its high sensitivity. For this purpose, the established ELISA method frequently used in the determination of analytes is suitable. In a preferred embodiment, the blood DNAM-1 concentration is determined by an ELISA method using a highly specific Tx25 monoclonal antibody. Further, it is preferable to use a kit that is designed to allow for rapid measurement by an ELISA method and ready acquisition of test results.

The test method of the invention not only determines the blood DNAM-1 concentration but also contributes to diagnosis of the probability of GVHD development. If a blood specimen taken from a test subject shows a high DNAM-1 concentration above the normal range, the data may be corroborative evidence indicating that the subject has or might have GVHD. In addition, the method described herein characteristically provides data for specifically predicting the probability of development of acute graft-versus-host disease (GVHD) or for estimating the risk of the development. The test results finally presented to a doctor are shown in an appropriate format for the doctor to predict onset of the disease.

Formats of test data

The test results may be shown in various formats. The data finally presented by the test method of the invention classify blood DNAM-1 concentrations through statistical analysis based on a normal range that is within Mean + 2SD calculated from control values of healthy individuals, and a concentration exceeding this range is evaluated to be abnormally high. Herein, "Mean + 2SD" means mean value + 2 x standard deviation, and only less than 2.5% of normal values exceed this range. Thus, if a blood DNAM-1 concentration is within the range, the concentration will be considered normal. As described later, the normal concentration of soluble DNAM-1 is not more than 0.098 nM. If the concentration is outside this normal range, it may be recommended to examine how much the concentration deviates from it and to take precautions against an increased probability of acute GVHD or to suspect that the disease has already developed.

The higher the blood DNAM-1 concentration, in other words, the more the concentration deviates from...
the normal range, the higher the tendency that acute GVHD will be developed in the future. However, it is dangerous to draw the conclusion with this result alone. In view of differences among individuals and variations by physiological conditions, it is necessary to obtain a pattern in which the blood DNAM-1 concentration is tracked over a time period.

[0043] Thus, the data may be preferably shown as a time-series profile of the blood DNAM-1 concentrations to allow for more objective diagnosis. Accordingly, the blood DNAM-1 concentration of a patient who has had hematopoietic stem cell transplantation from bone marrow or the like will be measured on days, namely, at a plurality of time points after the transplantation. From temporal changes of blood DNAM-1 concentration shown in the profile, the probability of acute GVHD development can be predicted. In other words, a sign of the disease onset can be predicted from a pattern of changes in blood DNAM-1 level. For example, if the blood DNAM-1 level shows a clear increasing tendency, it is reasonable to judge that the risk of the disease onset has increased.

In a more preferred embodiment of the time-series profile to support the above judgment or estimation, the time-series profile is represented in sections and the sections contain time points at least two of which have a higher DNAM-1 concentration than that of an immediately previous time point and which do not show a decreasing change. According to this preferred embodiment, alarming points are clearly presented when a doctor examines GVHD probability using the data provided by the method of the invention.

[0044] In the time-series profile, the statistical analysis may be performed by methods appropriately selected from various statistical methods and test methods used in the art. Preferably, a statistical analysis method suited for analyzing temporal changes in discrete data is used, and the significance of the statistical results and the degree of risk are presented.

The data may be presented in a manner such that an index is presented which links acute GVHD development and blood DNAM-1 concentration based on statistics. As an example, the criterion concentration in blood as an upper limit of normal range may be 0.098 nM. This concentration is used as a parameter defined in the border level shows a clear increasing tendency, it is reasonable to judge that the risk of the disease onset has increased.

[0046] Predicting the probability of acute GVHD development or estimating the risk of the development is to estimate the likelihood, preferably stochastically, whether GVHD is not developed and no noticeable subjective symptoms are seen, or whether although the condition is almost healthy, immunologic, metabolic or physiologic changes leading to the disease are still potential, or whether such changes have progressed and the disease will be developed in the future. This probability prediction or risk estimation is very significant in preventive medicine, in particular from the aspects of prognosis prediction and follow-up. These deeds form an aspect of diagnosis. The term "diagnosis" is a medical act in which a doctor determines whether a patient is diseased or not based on symptoms and various test results, or determines the form, pattern and stage of the disease to treat the patient. The test methods of the present invention provide the most useful data for such diagnosis acts, i.e., for predicting the probability of disease development or for estimating the risk of the development. The data having the aforementioned embodiments and presentation manners are based on pathological findings on immune system that DNAM-1 in blood as a monitoring marker is specifically associated with acute GVHD development. The data are statistically supported. Therefore, the data can be used directly for the prediction of acute GVHD development.

[0047] The risk of the disease onset will be estimated in practice by a doctor in consideration of the data provided by the test method of the invention together with other clinical data and situations of an individual patient (age, gender, anamnestic history and lifestyle). The estimation based on such information becomes even more reliable.

[0048] If a patient is diagnosed to have a high possibility of future acute GVHD development, the patient is kept under prognosis observation so that the patient can immediately receive appropriate treatment upon the onset of the disease. In this manner, even if GVHD is developed, the patient can be treated early. The data from the test methods of the invention are not only useful as a disease predicting marker specific for acute GVHD but also are significant in prognosis management after appropriate treatment of acute GVHD until remission. This is in a way retrospective from the standpoint of predicting and preventing acute GVHD development. In view of the finding that the blood DNAM-1 con-
centration characteristically changes such that it can be a disease predicting marker specific for acute GVHD, the data on the blood DNAM-1 concentration and changes thereof may be used as a monitoring marker that indicates pathological transition after treatment, or may be used as clinical data for evaluating therapeutic effects. Thus, the test methods of the invention include a test method that provides a time-series profile showing blood DNAM-1 concentrations that is used for prognosis management for a patient who is under treatment of acute graft-versus-host disease or that is used for determination of therapeutic effects.

Kits

[0049] The kit used in the present invention includes at least a Tx25 anti-DNAM1 monoclonal antibody, a human DNAM-1/FC chimeric protein and ELISA tools and is used to determine the blood DNAM-1 concentrations by performing an ELISA method using the Tx25 monoclonal antibody and create the time-series profile.

[0050] The kit contains various tools, materials and reagents required to perform the test method. Specifically, it contains the antibodies for the ELISA method, ELISA reagents, and reagents for determining the soluble DNAM-1 concentration in serum. The reagents include enzymes, standard substances for creating calibration curves, buffer solutions and washing solutions. The kit may also contain a set of required tools such as a microtiter plate for testing multiple specimens together. The specimen tested with the kit is preferably serum. In a high-throughput embodiment of the test method according to the present invention, the kit may contain a microreactor tool, specifically a chip-shaped tool. In this constitution, a system is preferably adopted in which signals from the chip (semi) automatically analyzing the specimen are captured and finally transformed to be in the form of numerical values in an electronic file and the file is saved to a predetermined directory on a computer, whereby the numerical data are statistically processed to allow for estimation of the probability of disease development. For the data processing, the data may be subjected to required correction or normalization and then be processed using appropriate software capable of statistic analysis. The data processing system may be created by a skilled person using existing technology, methods and procedures.

DNAM-1 neutralizing antibody and use thereof in GVHD treatment

[0051] Also described herein are GVHD treating or preventing pharmaceutical preparations (or pharmaceutical compositions) and GVHD molecular targeted therapy wherein an anti-DNAM-1 antibody or an analogue thereof is used as a DNAM-1 neutralizing antibody. The anti-DNAM-1 antibody that is an active component of the pharmaceutical preparations is preferably a monoclonal antibody.

Molecular targeted therapy for DNAM-1

[0052] Because the soluble DNAM-1 level is elevated when acute GVHD occurs, it can be safely said that the soluble or membrane DNAM-1 is involved in some way in acute GVHD. It was then assumed that DNAM-1 would be useful as a molecular target for prevention and treatment of acute GVHD. To develop methods and pharmaceutical compositions for treating or preventing GVHD, the present inventors studied molecular targeted therapy for DNAM-1 using murine GVHD models. In detail, they cloned DNAM-1 mouse homologous genes for the first time in the world (Tahara-Hanaoka, et al., Biochem Biophys Res Commun, 2005) to establish knockout mice, and prepared a neutralizing antibody for murine DNAM-1 (Tahara-Hanaoka, et al., Blood, 2006).

[0053] Researches using the neutralizing antibody for murine DNAM-1 demonstrated that: immunocytes involved with alloantigen specific immune response or inflammatory response related thereto are activated in acute GVHD to thereby excrete soluble DNAM-1; involvement of soluble or membrane DNAM-1 in some way in acute GVHD is strongly indicated; and acute GVHD can be prevented or treated by administering the anti-DNAM-1 antibody, as described in Example 4 later.

[0054] Of more than 20 clones of anti-DNAM-1 monoclonal antibody prepared by the present inventors, TX42 was the only neutralizing antibody. As this result indicates, preparing DNAM-1 neutralizing antibodies in acute GVHD is not easy. It should be also noted that the TX42 antibody destroys only the function of anti-DNAM-1 without reducing the number of DNAM-1 expressing cells by ADCC (antibody-dependent cell-mediated cytotoxicity) in vivo. Many of the antibodies induce ADCC to reduce the number of cells expressing the target molecule, resulting in frequent side effects such as weakened immune systems. In view of this too, the neutralizing antibodies such as the TX42 antibody having the above properties are advantageous anti-DNAM-1 antibodies for the prevention or treating methods as described herein.

[0055] Based on this, a molecular targeted therapy is disclosed wherein DNAM-1 in blood of a GVHD patient or a recipient that is a possible patient is used as a target molecule and GVHD is treated or prevented by administering an anti-DNAM-1 antibody or an analogue thereof that is a neutralizing antibody. Here, the words "neutralizing antibody" refer to an antibody molecule that specifically binds to the target DNAM-1 molecule and has activity to suppress or destroy biological activity of DNAM-1 (in particular, physiological activity associated with GVHD development or pathological conditions) in vivo. It is assumed that in the presence of excessive DNAM-1, the neutralizing antibody specifically binds to DNAM-1 involved in the adhesion of donor T cells in a graft with white blood cells of a recipient and thereby interferes with the action of DNAM-1, so that the cell-mediated cytotox-
icy is prevented from being induced to the T cells. By administering the anti-DNAM-1 antibody or an analogue thereof at a stage in which the blood DNAM-1 level starts to rise based on the test results, GVHD can be prevented or treated early.

Pharmaceutical preparations for treating or preventing GVHD

[0056] The pharmaceutical preparations (or pharmaceutical compositions) for use in treating or preventing GVHD described herein contain a DNAM-1 neutralizing antibody that is an anti-DNAM-1 antibody. For use as the anti-DNAM-1 antibodies, various types may be prepared by known techniques. When the pharmaceutical preparation is used for human, a preferred embodiment is as follows. The anti-DNAM-1 antibody is a humanized DNAM-1 antibody that is expressed by a human DNAM-1 gene introduced in a DNAM-1 knockout mouse as a mouse, rabbit or chicken may be used. The antibody that is immunoreactive preferably undergoes immune reaction specifically with DNAM-1, and may be a polyclonal antibody or a monoclonal antibody. In particular, monoclonal antibodies are preferable in view of properties such as antigen specificity, antigen affinity, neutralizing activity and cross reactivity. To prevent a side effect in which an antibody administered to a patient is recognized as a foreign protein and an immune response thereto is evoked, antibody humanization technology and techniques for producing humanized antibodies or monoclonal antibodies are used. Established and known methods may be used herein. For example, chimeric antibodies or humanized monoclonal antibodies may be prepared by techniques as described in Proc. Natl. Acad. Sci. USA (1983), 80, 7308-7312 by Teng, et al., and Immunology Today (1983), 4 (3), 72-79 by Kozbor, et al. Existing antibody producing cell lines such as CHO (Chinese hamster ovary) cells may be used for the production of antibodies.

[0057] The antibodies produced as described above may be subjected to appropriate modifications and alterations (fragmentation, complex formation with polypeptides or the like, modification for amino acid residues) as required while maintaining the ability as neutralizing antibodies. These variants obtained for the purpose of molecular stabilization, activity enhancement or controlling the half-life in blood are defined as analogues of the antibodies. These antibodies may also be used in the aforementioned test methods.

The pharmaceutical preparations of the invention may contain at least a pharmaceutically acceptable excipient, carrier or stabilizing agent, and may further contain a medicinal substance as an effective component for GVHD treatment. The medicinal substances include conventional medical agents for GVHD treatment such as steroids and immunosuppressive agents, and may further include substances capable of suppressing excessive expression of DNAM-1 genes. Pharmaceutically acceptable additives used may be, in addition to examples including substances conventionally used in the pharmaceutical industry, effectors such as macrophage-activating factors, substances enhancing stability or targeting ability of the anti-DNAM-1 antibody, and substances improving the half-life in blood of the antibody. The pharmaceutically acceptable carriers and excipients are known to the skilled person and include salts, sugars, other osmoregulators, buffering agents, acids, bases, other pH adjusters, viscosity improvers and colorants.

[0058] The forms of the preparations that are antibody drugs are not limited, and the preparations may be provided in the forms of freeze-dried preparations, powder preparations, solution preparations with pH-adjusted buffer solutions, and injectable microcapsule preparations. In the case of injectable preparations as an example, the antibody may be added together with required or desirable excipients and/or additives to a biocompatible or physiologically acceptable liquid carrier (preferably aqueous carrier) to give an injectable or infusible suspension or dispersion. These additional components may be added based on conventional formulating technology in the art. For example, they may be dissolved or suspended in a pharmaceutically acceptable nontoxic carrier such as saline or commercially available injectable distilled water such that the concentration will be 0.1 μg antibody/ml carrier to 10 mg antibody/ml carrier.

Described herein is also a use of an anti-DNAM-1 antibody in production of a pharmaceutical preparation for use in treating or preventing GVHD by administering the anti-DNAM-1 antibody or an analogue thereof that is a neutralizing antibody to blood DNAM-1 of a GVHD patient or a recipient that is a possible patient, as a target molecule. Uses The pharmaceutical preparation is administered to a patient in the form of an injectable preparation or an instillation preparation, specifically via intravascular administration or intravenous administration, in both cases of treating and preventing GVHD. Appropriate doses, concentrations, administration routes, timing and formulation may be individually determined by a doctor in consideration of symptoms and conditions of the patient in relation to the above GVHD treating or preventing methods. The doses may vary depending on patient conditions, administration purposes (whether it is for treatment or preventive) or types of the active components (e.g., the antibodies, the medicinal substances). For an adult, the preparation may be generally administered in the range of 10 μg to 1000 mg, and preferably 10 μg to 500 mg per administration. In the case of an injectable preparation, the preparation may be administered to a human patient one to several times in a day at a ratio of 1 μg to 100 mg, and preferably 50 μg to 50 mg per kg of the patient in each administration.
BRIEF DESCRIPTION OF THE DRAWINGS

[0059]

Fig. 1 indicates calibration curves for determination of DNAM-1 concentration by ELISA method wherein the calibration curve (A) is drawn based on DNAM-1 concentrations ranging from 0.015625 nmol to 2 nmol and the calibration curve (B) is created based on DNAM-1 concentrations from 0.015625 nmol to 0.5 nmol. The vertical axis shows soluble DNAM-1 concentration and the horizontal axis shows OD_{405nm} value.

Fig. 2 shows soluble DNAM-1 concentrations in blood at an initial onset of various blood diseases. The blood diseases in the figure are described in the specification. "Control" is a standard and provides a normal range.

Fig. 3 shows soluble DNAM-1 concentrations in blood after allogeneic transplantation and autologous transplantation.

[0060] The figure compares peak values of subjects clinically diagnosed to have acute GVHD, indicated by • plots, and of subjects clinically suspected to have acute GVHD, indicated by • plots.

Fig. 4 shows a correlation between acute GVHD occurrence and soluble DNAM-1 concentration. The figure compares peak values of soluble DNAM-1 concentration between acute GVHD positive and acute GVHD negative after allogeneic transplantation. The upper and lower dotted lines are drawn at 0.0983 and -0.0375, respectively. Fig. 5 indicates three patterns of temporal changes in blood DNAM-1 concentration based on test results with three subjects. The negative values are as a result of data processing in the ELISA method (subtraction of the blank).

Fig. 6-1 illustrates post-treatment changes in blood DNAM-1 concentration of two patients (YU and NK) who were transplanted with allogeneic bone marrow hematopoietic stem cells from unrelated donors and were complicated with acute GVHD. In the figure, "PSL" and "mPSL" mean methylprednisolone, "Alp" serum alkaline phosphatase, "Pulse" pulse treatment, and "MMF" mycophenolate mofetil. The negative values are as a result of data processing subtracting the blank in the ELISA method.

Fig. 6-2 shows changes in blood DNAM-1 concentration of a patient (YM) who was transplanted with allogeneic hematopoietic stem cells from an unrelated donor, over phases before and during acute GVHD development and after treatment. Indications and symbols are the same as in Fig.6-1.

Fig. 7 is a graph showing involvement of DNAM-1 by experiment using GVHD models. 1.0 Gy-irradiated C57BL6 mice were transplanted with spleen cells from BALB/c mice to prepare murine acute GVHD models. Some recipient mice had been preliminarily administered with an anti-DNAM-1 neutralizing antibody (TX42).

BEST MODE FOR CARRYING OUT THE INVENTION

[Examples]

[0061] Material sources, numerical conditions such as reagent concentrations and amounts, treating time and treating temperature, and treating methods used in Examples are no more than preferred example. Thus, it should not be construed that Examples limit the scope of the present invention.

Example 1

Correlation between blood diseases and soluble DNAM-1

[0062] The soluble DNAM-1 level in blood was measured to determine whether DNAM-1 was involved in the development of acute GVHD and whether or not DNAM-1 would increase at an initial onset of various blood diseases. To determine the soluble DNAM-1 concentration in serum, ELISA method was used.

Subjects and specimens

[0063] Clinical specimens were collected from 39 individuals (18 GVHD-positive transplant patients and 21 GVHD-negative transplant patients). In detail, serum from patients who received hematopoietic stem cell transplantation at Tsukuba University Hospital since November, 2001 was preserved at -70°C with the consent of the patients and was used as specimens in the test. Serum from a transplantation donor was used as a healthy control.

Reagents and antibodies

[0064] Tx25 hybridoma is a cell developed by one of the inventors of the present invention. Tx25 hybridoma was inoculated in the peritoneal cavity of a BALB/c-nu female mouse (6 weeks old), and the ascitic fluid was collected. Thereafter, Tx25 monoclonal antibody was isolated using MAb Trap kit purchased from Amersham Bioscience (Uppsala, Sweden).

[0065] Recombinant human DNAM-1/FC chimeric protein and biotinylated anti-human DNAM-1 polyclonal antibody were purchased from R&D System (Minneapolis, MN), and ExtrAvidin peroxidase (including a mouse ExtrAvidin peroxidase staining kit) and Fast o-phenylenediamine dihydrochloride (OPD) tablets were purchased from SIGMA-ALDRICH (Saint Louis, MO).

[0066] Buffer solutions used were PBS which con-
tained 8000 mg NaCl, 200 mg KCl, 1150 mg NaHPO₄ and 200 mg KH₂PO₄ in 1000 ml, and T-PBS which was prepared by mixing 1000 ml of PBS with 500 μg of Tween 20®.

ELISA method

[0067] The Ttx25 antibody diluted with PBS to 8 μg/ml was applied to an ELISA 96 well (12 x 8) flat-bottom microplate (DYNEX TECHNOLOGIES, Chantilly, VA) at 100 μl/well and was immobilized at 4°C overnight. The microplate was then washed with PBS three times each for 5 minutes, and blocking was performed with 1% BSA/PBS for 1 hour at room temperature. The microplate was washed with PBS two times each for 3 minutes. The wells in the first line were charged with 100 μl/well of the human DNAM-1/Fc chimeric protein standard in an eight-step dilution series from 2 nM prepared with PBS containing 0.05% Tween 20 (T-PBS). The wells in the second and later lines received 100 μl/well of patient serum. The microplate was left for 4 hours at room temperature and was washed with T-PBS three times each for 10 minutes. The wells were then applied with 100 μl/well of the biotinylated anti-human DNAM-1 antibody diluted with T-PBS to 0.6 μg/ml, and were left for 1 hour at room temperature. The microplate was washed with T-PBS three times each for 10 minutes. ExtrAvidin peroxidase diluted 250 times with T-PBS was added at 100 μl/well, and then the microplate was left for 30 minutes at room temperature and was washed with T-PBS three times each for 10 minutes. The peroxidase enzyme activity was measured at OD 405 nm using OPD as a chromogenic substrate.

[0068] In the eight-step dilution series of DNAM-1, the data obtained at from 0.0156 nM to 0.5 nM, a range thought to reflect the practical soluble concentration in patient serum, were used to create a calibration curve (Fig. 1). The soluble DNAM-1 concentrations in serum were determined based on the calibration curve.

Results for various blood diseases

[0069] "Mean + 2SD (mean value + 2 x standard deviation)" was calculated from control values of healthy individuals and was defined as a normal range. When the concentration exceeded this range, it was evaluated to be abnormally high. The normal concentration of soluble DNAM-1 was not more than 0.098 nM.

[0070] As indicated in Fig. 2, although only one specimen had a concentration far out of the normal range in Castleman’ s disease, the soluble DNAM-1 concentrations in serum of all the patients were found in the normal range in ALL (acute lymphocytic leukemia), AML (acute myelogenous leukemia), NHL (non-Hodgkin lymphoma; "diffuse large B"; "follicular"; "mantle cell"), HD (Hodgkin disease), MM (multiple myeloma) and ITP (immune thrombocytopenic purpura).

Results for acute GVHD

[0071] The soluble DNAM-1 concentration was measured after allogeneic transplantation possibly complicated with acute GVHD and autologous transplantation. In autologous transplantation, a patient is transplanted with frozen bone marrow or peripheral hematopoietic stem cells collected from himself and is not assumed to be complicated with acute GVHD.

[0072] As a result, 90% of the serum specimens of the autologous transplantation patients were found within the normal range but 45.7% of the serum specimens of the allogeneic transplantation patients exceeded the normal range as indicated in Fig. 3.

[0073] The above results show that the soluble DNAM-1 concentration in blood does not increase at an initial onset in many of the blood diseases but does specifically increase in acute GVHD. This will indicate involvement of DNAM-1 in some way in the process in which donor T cells damage recipient cells. 85.7% of the allogeneic transplantation patients who showed a high level of soluble DNAM-1 had been complicated with acute GVHD.

[0074] Fig. 4 compares the specimens of the allogeneic transplantation patients between acute GVHD positive and acute GVHD negative. As shown, 80% of the acute GVHD negative patients had a soluble DNAM-1 concentration in the normal range, whereas 58.1% of the acute GVHD positive patients had a concentration exceeding the normal range.

Thus, it can be safely said that when the soluble DNAM-1 concentration in blood exceeds the normal range, acute GVHD has developed, will soon develop, or can possibly develop.

Example 2

Examples of data presentation

[0075] Fig. 5 indicates three patterns of temporal changes in blood DNAM-1 concentration based on test results with three patients. The graphs of patients YT and TO contain time points at least two of which have a higher DNAM-1 concentration than that of an immediately previous time point and which do not show a decreasing change. Contrastingly in the pattern of KM, there are no parts to be sectionalized in such manner.

Example 3

Changes in blood DNAM-1 concentration after treatment of acute GVHD patients

[0076] Temporal changes in blood DNAM-1 concentration after initiation of treatment were examined with respect to hematopoietic stem cell transplantation patients complicated with acute GVHD. The blood DNAM-1 concentration was followed starting from "Day 0" when acute GVHD was diagnosed and treatment was started.
The blood DNAM-1 concentration was measured by the ELISA method as described in Example 1. Fig. 6-1 follows temporal changes in blood DNAM-1 concentration of two acute GVHD patients. Fig. 6-2 shows a case in which the probability of acute GVHD occurrence could not be accurately predicted from changes in blood DNAM-1 concentration after hematopoietic stem cell transplantation and consequently the patient developed acute GVHD and was treated. Each figure also indicates observed symptoms. The results in the figures show that the blood DNAM-1 concentration after initiation of the treatment, and the transition thereof until remission are useful as monitoring markers indicating transition after treatment or as clinical data for determination of treatment effects.

**Example 4**

DNAM-1 molecular targeted therapy with murine GVHD models

**[0078]** DNAM-1 mouse homologous genes were cloned (Tahara-Hanaoka, et al., Biochem Biophys Res Commun, 2005) and knockout mice were established (unpublished). A neutralizing antibody for the murine DNAM-1 was prepared (Tahara-Hanaoka, et al., Blood, 2006).

**[0079]** To demonstrate the neutralizing activity of the antibody, acute GVHD models were prepared by transplanting C57BL/6 recipient mice (1.0 Gy-irradiated) with allogeneic bone marrow from BALB/C donor mice. Of the murine GVHD models, the C57BL/6 mice transplanted with bone marrow cells from DNAM-1 gene knockout mice (BALB/C background) were noticeably prevented from acute GVHD onset (Fig. 7).

**[0080]** Next, murine acute GVHD models were prepared by transplanting sublethally irradiated (0.4 Gy), BALB/C x C57BL/6 F1 mice with spleen cells from BALB/C mice. The recipient mice that had been preliminarily administered with the anti-DNAM-1 neutralizing antibody (TX42) were markedly prevented from acute GVHD onset (Fig. 8).

**[0081]** The above results strongly suggested that immunocytes involved in alloantigen specific immune response or inflammatory response related thereto were activated in acute GVHD to excrete soluble DNAM-1, and soluble or membrane DNAM-1 was in some way involved in acute GVHD. Further, acute GVHD was shown to be preventable or treatable by administering the anti-DNAM-1 antibody (TX42).

### Claims

1. A test method for predicting acute graft-versus-host-disease (GVHD) development comprising determining the soluble DNAM-1 concentration in blood collected from a transplant patient and examining temporal changes of said concentration wherein the soluble DNAM-1 concentrations in blood are classified according to a normal range that is within Mean + 2SD from control values of healthy individuals, and a concentration exceeding this range is evaluated to be abnormally high.

2. The test method according to claim 1, wherein the soluble DNAM-1 concentrations in blood are shown as a time-series profile.

3. The test method according to claim 2, wherein the time-series profile is shown in sections and the sections contain time points at least two of which have a higher soluble DNAM-1 concentration than that of an immediately previous time point and which do not show a decreasing change.

4. The test method according to any one of claims 1 to 3, wherein the transplant patient is a bone marrow or peripheral, hematopoietic stem cell transplantation patient.

5. The test method according to any one of claims 1 to 4, wherein the soluble DNAM-1 concentrations in blood are determined from a patient who has had allogeneic hematopoietic stem cell transplantation and are used to help prognosis management.

6. The test method according to claim 2, wherein the time-series profile showing soluble DNAM-1 concentrations in blood is used for prognosis management for a patient who is under treatment of acute graft-versus-host disease or for determination of therapeutic effects for acute GVHD.

7. The test method according to any one of claims 1 to 6, wherein the soluble DNAM-1 concentration in blood is determined by ELISA method using a Tx25 anti-DNAM-1 monoclonal antibody.

8. Use of a kit comprising at least a Tx25 anti-DNAM-1 monoclonal antibody, a human DNAM-1/Fc chimeric protein and an ELISA tool wherein said kit is used to determine the soluble DNAM-1 concentrations in blood by performing an ELISA method using the Tx25 anti-DNAM-1 monoclonal antibody for creating the time-series profile described in claim 2 or 6 in order to predict acute GVHD development as claimed in claim 2 or 6.

9. A pharmaceutical preparation for use in a method of
treating or preventing acute GVHD, comprising an anti-DNAM-1 neutralizing antibody.

10. The pharmaceutical preparation according to claim 9, wherein the anti-DNAM-1 antibody is a monoclonal antibody.

11. The pharmaceutical preparation according to claim 9 or 10, wherein the anti-DNAM-1 antibody is a humanized DNAM-1 antibody.

12. The pharmaceutical preparation according to any one of claims 9 to 11, wherein the pharmaceutical preparation further comprises at least a pharmaceutically acceptable excipient, carrier or stabilizing agent, and may further comprise a medicinal substance for acute GVHD treatment.

13. A use of an anti-DNAM-1 neutralising antibody in the production of a pharmaceutical preparation for use in treating or preventing acute GVHD.

Patentansprüche

1. Testverfahren zum Vorhersagen einer akuten Transplantat-Wirt-Erkrankungsentwicklung (GVHD-Entwicklung), wobei das Testverfahren das Bestimmen der Konzentration von löslichem DNAM-1 in von einem Transplantationspatienten gesammeltem Blut und das Untersuchen zeitlicher Änderungen der Konzentration umfasst, wobei die Konzentrationen von löslichem DNAM-1 im Blut in Übereinstimmung mit einem Normalbereich, d. h. innerhalb Mittelwert + 2SD gegenüber Kontrollwerten gesunder Personen, klassifiziert werden und eine Konzentration, die diesen Bereich übersteigt, als anomalo hoch bewertet wird.

2. Testverfahren nach Anspruch 1, bei dem die Konzentrationen von löslichem DNAM-1 im Blut als ein zeitreihenprofil gezeigt werden.

3. Testverfahren nach Anspruch 2, bei dem das zeitreihenprofil in Abschnitten gezeigt wird und die Abschnitte Zeitpunkte enthalten, von denen wenigstens zwei eine höhere Konzentration von löslichem DNAM-1 als ein unmittelbar vorausgehender Zeitpunkt aufweisen und die keine Abnahmeänderung zeigen.

4. Testverfahren nach einem der Ansprüche 1 bis 3, bei dem der Transplantationspatient ein Knochenmarkstransplantationspatient oder ein Patient ist, dem periphery blutbildender Stammzellen transplantiert worden sind.

5. Testverfahren nach einem der Ansprüche 1 bis 4, bei dem die Konzentrationen von löslichem DNAM-1 im Blut von einem Patienten bestimmt werden, der eine Transplantation allogenetischer blutbildender Stammzellen erfahren hat, und als Hilfe für das Prognosemanagement verwendet werden.


7. Testverfahren nach einem der Ansprüche 1 bis 6, bei dem die Konzentration von löslichem DNAM-1 im Blut durch das ELISA-Verfahren unter Verwendung eines monoklonalen TxAnti-DNAM-1-Antikörpers bestimmt wird.


10. Arzneimittelzubereitung nach Anspruch 9, bei der der Anti-DNAM-1-Antikörper ein monoklonaler Antikörper ist.

11. Arzneimittelzubereitung nach Anspruch 9 oder 10, bei der der Anti-DNAM-1-Antikörper ein humanisierter DNAM-1-Antikörper ist.

12. Arzneimittelzubereitung nach einem der Ansprüche 9 bis 11, bei der die Arzneimittelzubereitung ferner wenigstens einen pharmazeutisch akzeptablen Exzipienten, Träger oder Stabilisator umfasst und ferner eine medizinische Substanz für die Behandlung akuter GVHD umfassen kann.

13. Verwendung eines neutralisierenden Anti-DNAM-1-Antikörpers bei der Herstellung einer Arzneimittelzubereitung zur Verwendung bei der Behandlung oder Verhinderung akuter GVHD.
Revendications

1. Procédé de test permettant de prédire le développement de la maladie aigue du greffon contre l'hôte (GVHD) comprenant la détermination de la concentration de DNAM-1 soluble dans le sang prélevé d'un patient transplanté et l'examen de variations temporelles de ladite concentration, les concentrations de DNAM-1 soluble dans le sang étant classifiées conformément à un domaine normal de variations se situant entre la moyenne et + 2 écarts-types de valeurs de contrôle d'individus en bonne santé et une concentration excédant ce domaine de variations est évaluée comme anormalement élevée.

2. Procédé de test suivant la revendication 1, dans lequel les concentrations de DNAM-1 soluble dans le sang sont présentées comme un profil de séries chronologiques.

3. Procédé de test suivant la revendication 2, dans lequel le profil de séries chronologiques est présenté en sections et les sections contiennent des moments spécifiques dont au moins deux ont une concentration de DNAM-1 soluble supérieure à celle d'un moment spécifique immédiatement antérieur et qui ne montre pas de variation décroissante.

4. Procédé de test suivant une quelconque des revendications 1 à 3, dans lequel le patient transplanté est un patient transplanté de cellules souches hématopoïétiques de la moelle osseuse ou du sang périphérique.

5. Procédé de test suivant une quelconque des revendications 1 à 4, dans lequel sont déterminées les concentrations de DNAM-1 soluble dans le sang d'un patient ayant subi une transplantation allogénique de cellules souches hématopoïétiques et qui sont utilisées pour aider à la gestion des pronostics.

6. Procédé de test suivant la revendication 2, dans lequel le profil de séries chronologiques présentant des concentrations de DNAM-1 soluble dans le sang est utilisé pour la gestion des pronostics pour un patient sous traitement de la maladie du greffon contre l'hôte ou pour déterminer les effets thérapeutiques dans le cas d'une GVHD aiguë.

7. Procédé de test suivant une quelconque des revendications 1 à 6, dans lequel la concentration de DNAM-1 soluble dans le sang est déterminée suivant la méthode ELISA en utilisant un anticorps DNAM-1 monoclonal Tx25.

8. Emploi d'une trousse comprenant au moins un anticorps DNAM-1 monoclonal Tx25, une protéine chimérique DNAM-1/Fc humaine et un outil ELISA, ladite trousse étant utilisée pour déterminer les concentrations de DNAM-1 soluble dans le sang en exécutant la méthode ELISA en utilisant un anticorps DNAM-1 monoclonal Tx25 pour créer le profil de séries chronologiques décrit à la revendication 2 ou 6 afin de prédire le développement d'une GVHD aiguë, comme cela est revendiqué à la revendication 2 ou 6.

9. Préparation pharmaceutique destinée à être utilisée dans un procédé de traitement ou de prévention de la GVHD aiguë comprenant un anticorps anti-DNAM-1 neutralisant.

10. Préparation pharmaceutique suivant la revendication 9, dans laquelle l’anticorps anti-DNAM-1 est un anticorps monoclonal.

11. Préparation pharmaceutique suivant la revendication 9 ou 10, dans laquelle l’anti-anticorps anti-DNAM-1 est un anticorps DNAM-1 humanisé.

12. Préparation pharmaceutique suivant une quelconque des revendications 9 à 11, dans laquelle la préparation pharmaceutique comprend, en outre, au moins un excipient, porteur ou agent stabilisant pharmaceutiquement acceptable et peut, en plus, comprendre une substance médicale de traitement de la GVHD aiguë.

13. Utilisation d’un anticorps anti-DNAM-1 neutralisant dans la production d’une préparation pharmaceutique destinée à être utilisée dans le traitement ou dans la prévention de la GVHD aiguë.
Fig. 1

(A) (B)

Solubility DMAM - I (nm)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7

2.5 2 1.5 1 0.5 0 0.5 1 1.5 2

EP 2 012 123 B1
Fig.3
Fig. 5

- **HO(allo BMT)**
- **GvHD**

**KM**

**El(allo-BMT)**

**eDNA1-(GAA)**

**eDNA1-(sAA)**

**eDNA1-(mAA)**

**eDNA1-(mKM)**

**eDNA1-(mEl)**
Fig. 6.1

YU (ALL on disease, allo-BMT)

NK (Ph1+ ALL in 1st CR, allo-BMT)
REFERENCES CITED IN THE DESCRIPTION

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